DOCKET NO: 159820US55

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
ALAN K. SMITH, ET AL.	: EXAMINER: BELYAVSKI
SERIAL NO: 09/027,671	:
FILED: FEBRUARY 23, 1998	: GROUP ART UNIT: 1644
FOR: HUMAN LINEAGE COMMITTED CELL COMPOSITION WITH ENHANCED PROLIFERATIVE POTENTIAL, BIOLOGICAL EFFECTOR FUNCTION, OR BOTH; METHODS FOR OBTAINING SAME; AND THEIR USES	:

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I, Douglas M. Smith hereby declare:

1. I am a named inventor on the above-identified application.

2. I have a Ph. D. from The University of Chicago in Immunology, 1987.

3. From 1996 to the present, I have been employed by Aastrom, the assignee of this application, and I am engaged in research and development in the fields of immunology, stem cell biology, tissue regeneration and repair.

4. The following experiments were performed by me or under my supervision and control.

5. These experiments show that human osteoclasts and osteoblasts are obtained with enhanced replicative potential when cultured under conditions of medium replacement when compared to cells cultured under more traditional static culturing methods.

6. The effect of frequent medium exchange on culture output of Tissue Repair Cells (TRCs) was evaluated in small-scale cultures under a precise range of conditions as claimed in the invention. Briefly, cultures were initiated by inoculation of ficoll-hypaque density gradient separated human bone marrow mononuclear cells (BM MNCs) at low (1.5×10^5 cells/cm^2), intermediate (3×10^5 cells/cm^2) or high (6×10^5 cells/cm^2) inoculum densities. Medium exchange was carried out under essentially continuous conditions at an average rate of 12.5%, 25%, 37.5% or 50% per day as indicated and as described in the above-identified application. These conditions enable continuous cell culture without removal, hemi-depletion or other loss of cells during the culture process. Conventional static cultures (0% exchange) were included at each inoculum density (low, intermediate or high) as a control. The cultures were harvested for determination of total viable cell count and flow cytometry on days 7, 12 and 19. Standard colony forming assays to enumerate colony forming unit-fibroblast (CFU-F) in semi-solid medium were determined only at the day 12 harvest time point. Results for the mean of n= 2-3 experiments are shown in each figure.

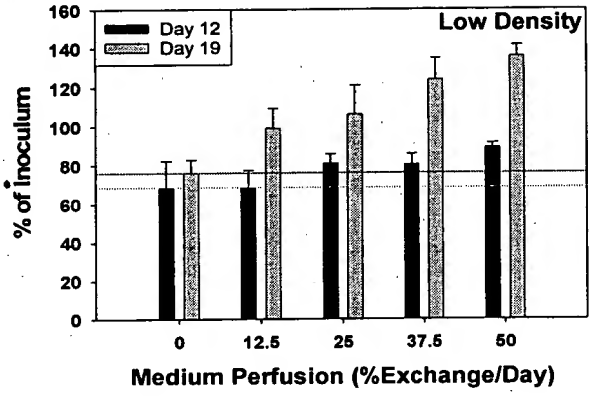
7. The results shown in Figure 1 (A-C) demonstrate a striking enhancement in the yield of total viable cells under conditions of frequent medium exchange (12.5 – 50% volume/volume exchange per day) when compared to static cultures (0% exchange). Results are expressed as the yield of harvested cells on days 12 or 19 of culture as a percentage of the starting inoculum. Three different inoculum densities (low, intermediate and high) were evaluated as described above.

8. The $CD14^+$ subset is a primary progenitor population for macrophages, dendritic cells and specifically osteoclasts which function in bone remodeling. As shown in Figures 2 and 3, the yield of $CD14^+$ cells delineated by the $CD14^+$ autofluorescent-high and $CD14^+$ autofluorescent-low subpopulations is enhanced by frequent medium exchange. Similarly, the yield of $CD90^+$ cells (Figure 4) is increased markedly over a range of medium exchange

rates when compared to static cultures (0% medium exchange). Significantly, the CD90 surface antigen (Thy-1) has been established as a key mesenchymal marker for osteoblast progenitors. This marker correlates closely with osteogenesis mediated by TRCs both in a murine xenogeneic model of human bone formation and in humans. In addition, CFU-F-forming activity, a key attribute directly associated with the $CD90^+$ population *and* bone formation also is enhanced with increasing rate of medium exchange (Figures 5-A and 5-B). Thus, the ability to form colonies in semi-solid methylcellulose is an excellent indicator of $CD90^+$ cell post-harvest proliferative capacity and biological activity.

9. These experiments demonstrate a direct relationship between culture output and medium exchange rate with time in culture. In other words, these data demonstrate that under the various medium replacement conditions used, osteoblasts and osteoclasts were obtained that had enhanced replicative potential when compared to statically cultured cells, particularly shown by the increased yield of total viable cells obtained. Moreover, these observations strongly support the claims for enhanced replicative potential and biological function of osteoblasts for bone formation and associated cell types such as osteoclast progenitors which function in bone remodeling.

A.



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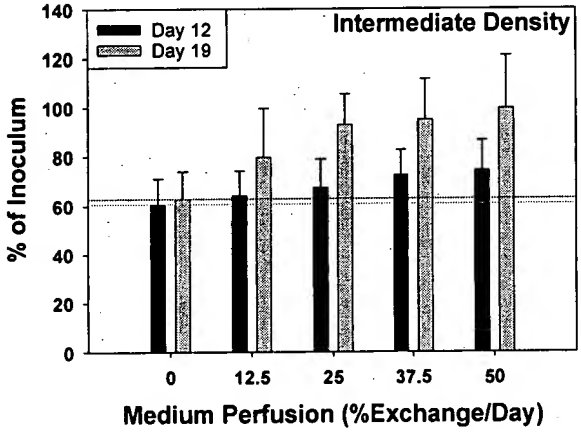

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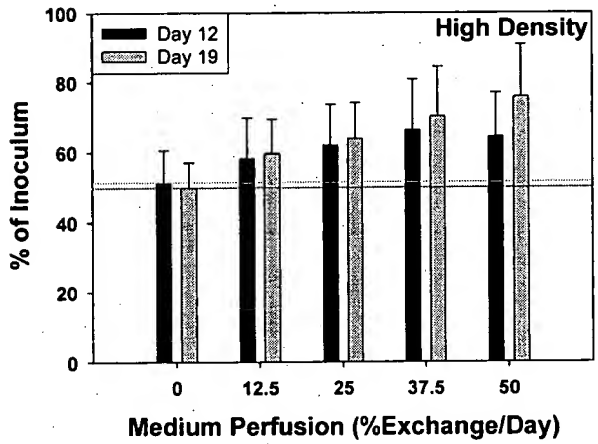
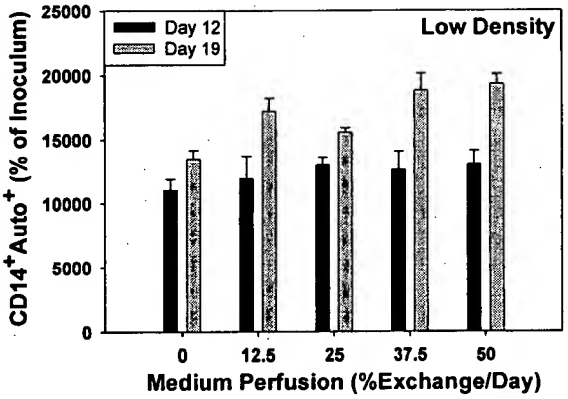


Figure 1. Frequent medium exchange enhances the output of total viable cells in bone marrow cultures. A) Low inoculum density (1.5 x 10^5 cells/cm^2); B) Intermediate inoculum density (3 x 10^5 cells/cm^2); and C) High inoculum density (6 x 10^5 cells/cm^2).

A.



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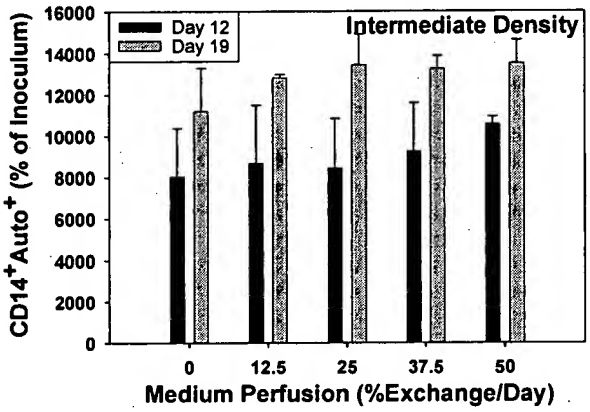

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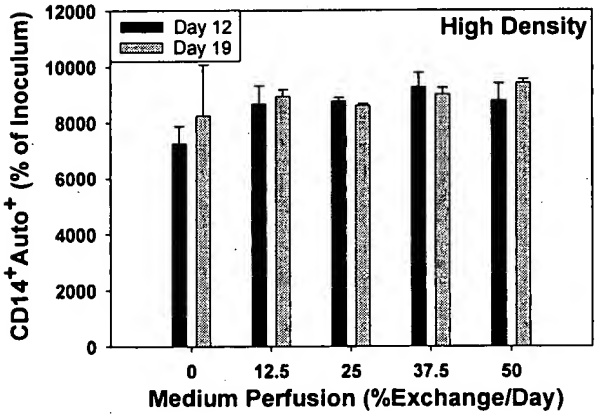
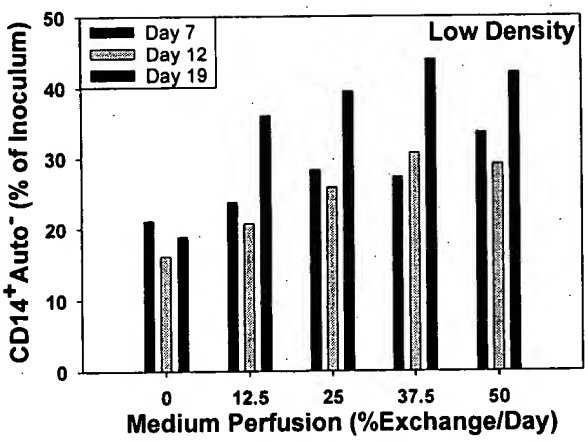


Figure 2. Frequent medium exchange enhances the output of $CD14^+$ (Autofluorescent-high) osteoclast progenitors in bone marrow cultures. A) Low inoculum density (1.5×10^5 cells/cm^2); B) Intermediate inoculum density (3×10^5 cells/cm^2); and C) High inoculum density (6×10^5 cells/cm^2).

A.



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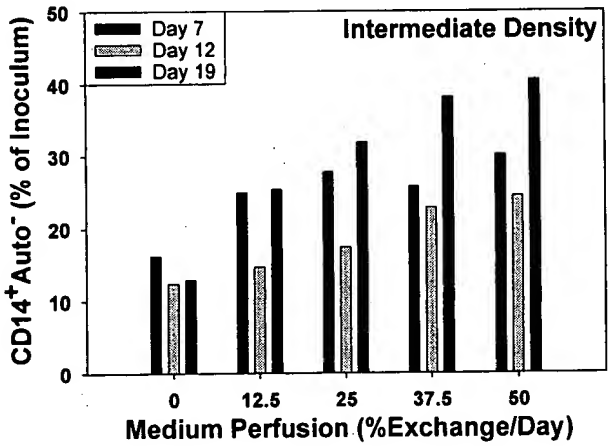

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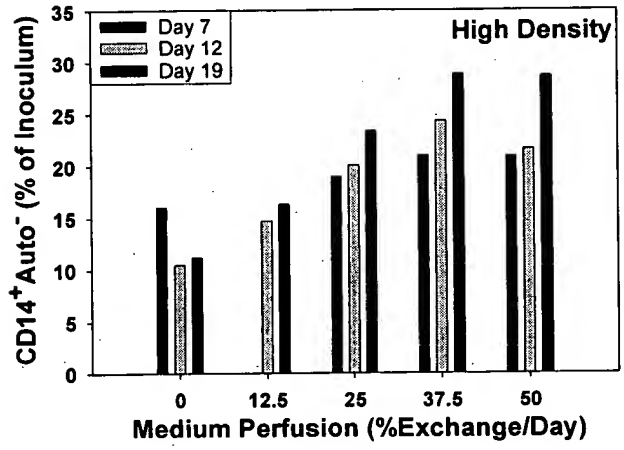
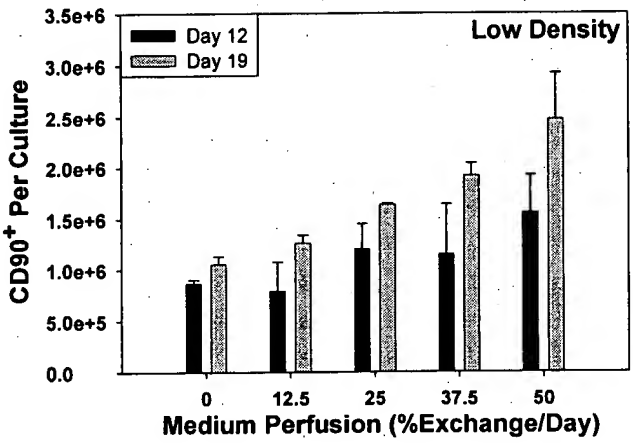


Figure 3. Frequent medium exchange enhances the output of $CD14^+$ (Autofluorescent-low) osteoclast progenitors in bone marrow cultures. A) Low inoculum density (1.5×10^5 cells/cm^2); B) Intermediate inoculum density (3×10^5 cells/cm^2); and C) High inoculum density (6×10^5 cells/cm^2).

A.



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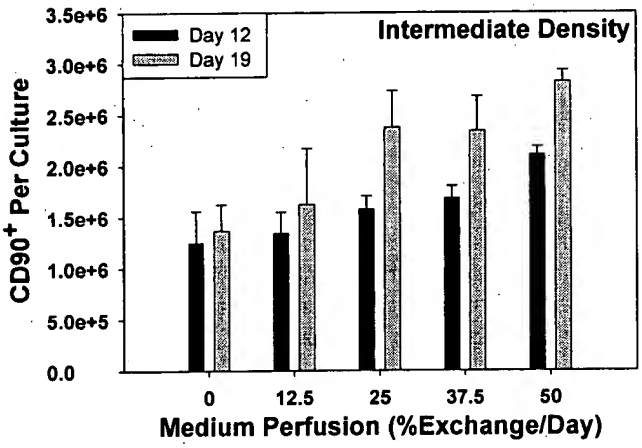

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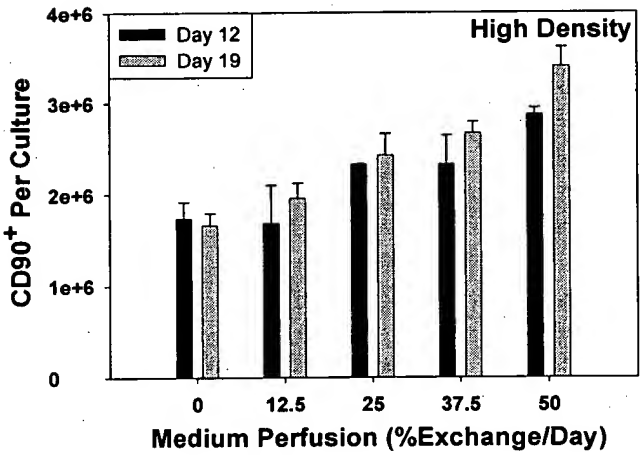
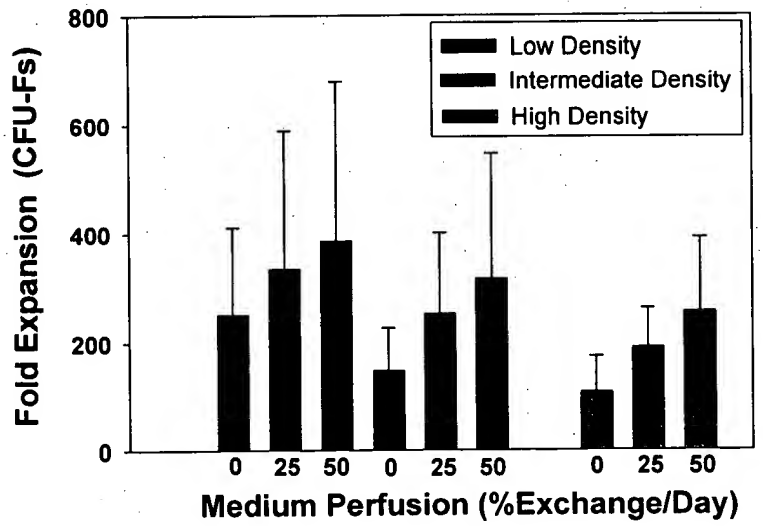


Figure 4. Frequent medium exchange enhances the output of $CD90^+$ mesenchymal/osteoblast progenitors in bone marrow cultures. A) Low inoculum density (1.5×10^5 cells/cm^2); B) Intermediate inoculum density (3×10^5 cells/cm^2); and C) High inoculum density (6×10^5 cells/cm^2).

A.



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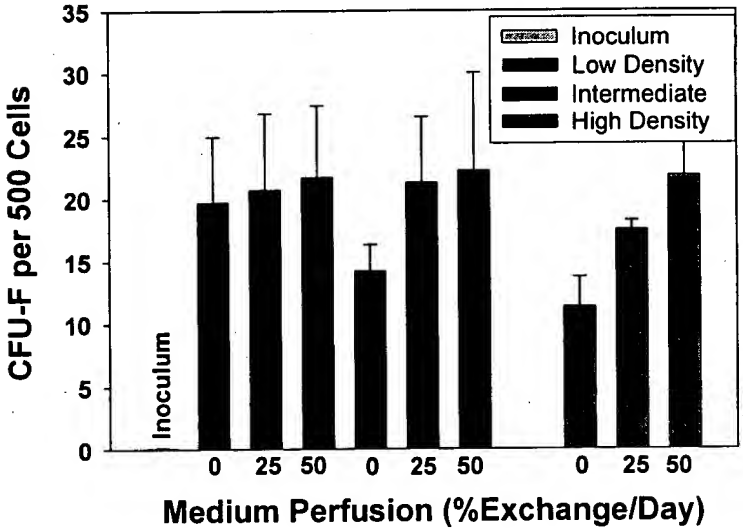

Figure 5. Frequent medium exchange enhances the output of colony forming unit-fibroblast (CFU-F) in bone marrow cultures at the time of harvest on day 12. A) Fold expansion of CFU-Fs at the time of harvest relative to inoculum; and B) Frequency of CFU-Fs per 500 harvested bone marrow cells (day 12).

10. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Douglas M. Smith

April 6, 2007
Date

DOCKET NO: 216499USCONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

ALAN K. SMITH, ET AL. : EXAMINER: BELYAVSKI, M

SERIAL NO: 10/668,214 :

FILED: SEPTEMBER 24, 2004 : GROUP ART UNIT: 1644

FOR: HUMAN LINEAGE COMMITTED CELL COMPOSITION WITH ENHANCED PROLIFERATIVE POTENTIAL, BIOLOGICAL EFFECTOR FUNCTION, OR BOTH; METHODS FOR OBTAINING SAME; AND THEIR USES

DECLARATION UNDER 37 C.F.R. §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Now comes Mr. Alan K. Smith who state that:

1. I along with Mr. Douglas M. Smith and Mr. Ramkumar K. Mandalam are the named inventors of the above-identified application for patent.

2. Mr. Douglas M. Smith, Mr. Ramkumar K. Mandalam, and I are authors, along with G.C Gorgas, T.C. Jensen, K.H. Hastie, and D.A. Brott of a meeting abstract for the American Society of Hematology, 39th Annual Meeting, December 5-9, 1997 published in the journal *Blood* on November 15, 1997.

3. The title of this meeting abstract is "Clinical scale expansion of dendritic cells in a continuously perfused bioreactor system."

4. As is customary in research, G.C Gorgas, T.C. Jensen, K.H. Hastie, and D.A. Brott were named as co-authors on this meeting abstract but did not contribute to the conception of the invention disclosed and claimed in the application.

5. G.C Gorgas, T.C. Jensen, K.H. Hastie, and D.A. Brott were working under our direction and supervision.

6. The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Alan K. Smith

Alan K. Smith

3/13/06

Date